Original Article

Expression of Human Epidermal Growth Factor Receptor (HER2/neu) and Proliferative Marker Ki-67 in Nonneoplastic, Preneoplastic, Neoplastic Lesions of Gallbladder and Its Association with Clinicopathological Parameters

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Abstract

Introduction: Globally, gallbladder carcinoma (GBC) ranks sixth among gastrointestinal tract tumors. Gallbladder cancer is difficult to diagnose. Nevertheless, there is a rising trend of gallbladder cancer; even then, chronic cholecystitis persists commonly among gallbladder lesions nursing various epithelial alterations, ultimately resulting in carcinoma. The current research is performed to evaluate the expression of HER2/neu (human epidermal growth factor receptor 2), Ki-67 in nonneoplastic, preneoplastic, neoplastic gallbladder lesions and to assess the association of expression of HER2/neu, Ki-67 with clinicopathological parameters in gallbladder lesions. **Materials and Methods:** A total of 76 cases were included in the study, out of which 19 cases were considered under neoplastic group (malignant as well as preneoplastic) and 57 cases (nonneoplastic) were considered under control group. Immunohistochemical staining results of HER2/neu and Ki-67 were evaluated. The correlation was noted among both groups. Statistical analysis was assessed utilizing MS Excel 2021 and SPSS V 25.0 software. The Chi-square test was utilized for evaluating association among variables. P <0.05 was considered statistically significant. A case–control hospital-based study was conducted from March 2022 to July 2024 (For 2 years). The ethical clearance was obtained with IEC number TMU/IEC/2024-25/007/12. **Results:** Positive HER2/neu expression (+2, +3) was noted in 26.3% (5/19) of malignant cases (neoplastic group), whereas the expression was completely absent in the nonneoplastic group (P < 0.05). Ki-67 labeling index (≥20%) expression was noted in 57.8% (11/19) of the neoplastic group (P < 0.05), while it was completely absent in the nonneoplastic group. **Conclusions:** HER2/neu and Ki-67 were overexpressed in neoplastic cases as compared with the control group. Moreover, HER2/neu can act as potential target therapeutic modality in GBC cases.

Keywords: Cholecystitis, gallbladder carcinoma, HER2 neu

Introduction

Gall bladder carcinoma (GBC) is an unusual neoplastic disease with bad prognosis. With an increasing trend in its occurrence and restricted therapeutic modalities, it inhabits sixth position among gastrointestinal tract tumors and accounts for 90%–95% of biliary tract malignancies. Affliction of GBC is noticed more commonly in females.[1] Risk factors include obesity and untreated chronic calculous cholecystitis. These risk factors exemplify carcinogenesis.[2] HER2, a cellular gene also called as c-erbB2, encodes thymidine kinase activity, which is

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robustly interconnected with EGFR.[3] EGFR overexpression is observed in GBC.[4] The objective of the current research was to assess immunohistochemical (IHC) expression of HER2/neu and Ki-67 in neoplastic gallbladder pathologies, comparing them nonneoplastic lesions (control group) and correlating them with various clinicopathological parameters.

Materials and Methods

A case–control hospital-based research was carried out from March 2022 to July 2024 (For 2 years). The ethical clearance was obtained with IEC number TMU/IEC/2024-25/007/12. The procedures used follow the guidelines laid down in the Declaration of Helsinki.

Inclusion criteria

Gallbladder specimens received in the pathology department reported as neoplastic lesions and preneoplastic were grouped under cases, whereas nonneoplastic lesions of gallbladder were included as controls.

Exclusion criteria

- Subjects with any concomitant malignancy (metastasis) or previous intervention (surgery/radiotherapy/chemotherapy)
- Specimens that have undergone autolysis
- Specimens without proper labels.

Sample size

The research included 76 cases with associated relevant clinical information. Cases and controls were classified into two groups: Neoplastic group: A total of 19 cases, this constituted GBC cases as well as pre-neoplastic cases such as chronic cholecystitis, mild , moderate and severe dysplasia. Other group was Nonneoplastic, which constituted 57 cases inclusive of chronic cholecystitis, chronic calculous cholecystitis, adenomatous hyperplasia, and eosinophilic, follicular, xanthogranulomatous cholecystitis were considered as control group. Cholecystectomy specimens received in histopathology section were selected in accordance with inclusion criteria and exclusion criteria. In accordance with laboratory protocol, overnight preservation and fixation in 10% neutral buffered formalin was done followed by grossing, specimens were processed along with H and E staining. Immunohistochemical staining: 4 μm sections were obtained and subjected to immunohistochemistry for Ki-67 and HER2/neu as per manufacturer's protocol (path-insitu). IHC antibody HER2 neu and Ki-67 source was Path-insitu with ready-to-use dilution was used with incubation period of one hour. Breast carcinoma cases were taken as positive controls for IHC evaluation. Ki-67 expression was evaluated by calculating the proportion of tumor cell nuclei that showed positive staining regardless of intensity of staining, with a threshold of 20% being considered positive [Figure 1]. The expression of HER2/neu was evaluated by scoring cell membrane staining. Scores of 0 and +1 were contemplated as negative and +2 and +3 were contemplated as positive[5] [Figure 2].

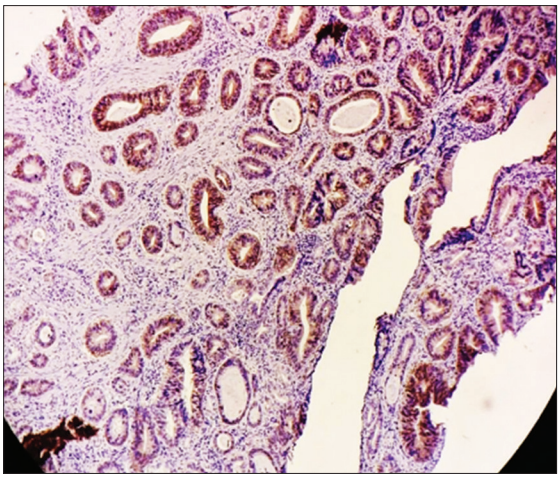

Figure 1: Poorly differentiated adenocarcinoma showing strong membranous (score + 3) positive immune reaction for HER2/neu marker (×100)

Statistical analysis

Information was gathered, entered, and assessed utilizing MS Excel 2021 and SPSS V 25.0 software (IBM, Chicago, USA). Frequencies and percentages were utilized to summarize qualitative characteristics. Statistical tests, both analytical and descriptive measures, were utilized to summarize quantitative variables. The Chi-square test was utilized for evaluating association among variables. P <0.05 was considered statistically significant.

Results

The research included 76 cases with associated relevant clinical information. Cases and controls were classified into two groups: Neoplastic group: Out of a total of 76 cases, 19 cases were observed in neoplastic group that constituted 9 (11.8%) cases of GBC, preneoplastic cases included 6 cases of chronic cholecystitis with mild dysplasia (7.8%), 2 (2.6%) cases of chronic cholecystitis with moderate dysplasia, and 2 (2.6%) cases of chronic cholecystitis with severe dysplasia. Other group was nonneoplastic: Which constituted 57 cases inclusive of 17 (22.4%) cases of chronic cholecystitis, 27 (35.5%) cases of chronic calculous cholecystitis, 2 (2.6%) cases of adenomatous hyperplasia with chronic cholecystitis, 5 (6.6%) cases of eosinophilic cholecystitis, 4 (5.3%) cases of xanthogranulomatous cholecystitis, and 2 (2.6%) cases of follicular cholecystitis were considered the control group. The average age of presentation was noted around 45 years of age with female predilection (73.7%), as depicted in Tables 1 and 2.

Immunohistochemical results

Positive HER2/neu expression (+2 and +3) was noted in 26.3% (5/19) of neoplastic cases, whereas it was entirely absent in nonneoplastic group (control group) (P = 0.001), as depicted in Table 3. Ki-67 positive expression: Ki-67 labeling index (LI) (≥20%) expression was noted in 57.8% (11/19) of neoplastic group (P = 0.001), while it was completely absent

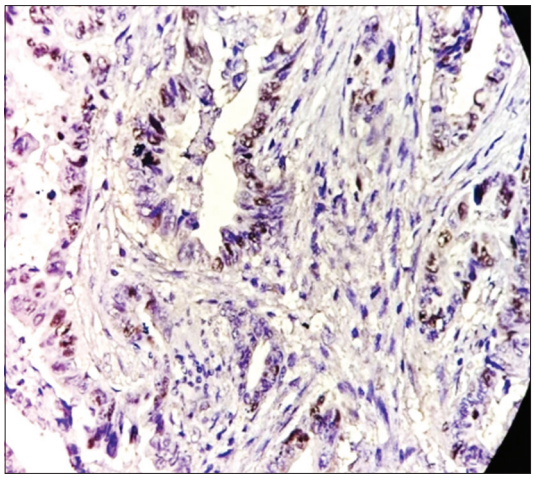

Figure 2: Poorly differentiated adenocarcinoma showing strong membranous Ki 67 positive >20% reaction for (×100)

in nonneoplastic group (control group), as depicted in Table 4. Correlation between HER2/neu and clinicopathological parameters in neoplastic group: HER2/neu overexpression was predominantly observed in elder subjects (P = 0.001) and in females (P = 0.001). HER2/neu overexpression was predominantly observed in subjects with presenting with weight loss. HER2/neu overexpression did not demonstrate a substantial significant difference with other clinical findings. Association of Ki-67 with clinicopathological parameters in neoplastic group: Ki-67 positive expression was noted in 57.8% (11/19) of neoplastic conditions. Ki-67 LI correlation was substantial with age (P = 0.001) and gender (P = 0.001). Ki-67 overexpression was prominently noted in subjects presenting with jaundice, while other clinical features showed no significant difference.

Discussion

GBC is an aggressive disorder with poorer prognosis with inefficacious therapeutic modalities. Hence, there is a requirement of efficacious therapeutic agents that will provide targeted treatment.[6] With respect to gender, gallbladder lesions were noted prominently in females, which was comparable to research done by Chaube *et al*.[7] HER2/neu, an EGFR family oncogene, has a vital role in formation of tumor in case of breast carcinoma. HER2/neu overexpression is also noted in various other solid malignancies.[8] In the current study, 26.3% (5/19) of GBC cases showed HER2/neu positive expression, and HER2/neu expression in the control group was completely absent. HER2/neu positive expression in GBC varies from 2% to 46.5% in accordance with research studies conducted by Nakazawa *et al*., Kawamoto *et al*., and Shafizadeh *et al*.[9-11] This variation is attributed to different methods utilized and variable HER2/neu scorning system utilized in different research. Research conducted by Chaube *et al*. and Puhalla *et al*.[7,12] had considered +2 and +3 score as positive similar to current research, whereas Roa *et al*. had accounted +3 to be HER2/neu positive.[13] Comparable findings to the current study were noted in research conducted by Pujani *et al*. in which HER2/neu positive expression was noted in 24% of GBC cases with entirely absent HER2/neu expression in the control group.[14] Furthermore, similar findings to the current research were noted in research performed by Yoshida *et al*., which showed HER2/

Table 1: Distribution of subjects with nonneoplastic gallbladder lesions based on histopathology

Diagnosis based on histopathology	Frequency (%)
Nonneoplastic	
Chronic cholecystitis	17 (22.4)
Chronic cholecystitis with cholelithiasis	27 (35.52)
Adenomatous hyperplasia with chronic cholecystitis	2 (2.6)
Eosinophilic cholecystitis	5 (6.6)
Xanthogranulomatous cholecystitis	4 (5.3)
Follicular chronic cholecystitis with cholelithiasis	2 (2.6)
Total	57

Table 2: Distribution of subjects with neoplastic gallbladder lesions based on histopathology

Diagnosis based on histopathology	Frequency (%)
Preneoplastic	
Chronic cholecystitis with mild dysplasia	6 (7.8)
Chronic cholecystitis with moderate dysplasia	2 (2.6)
Chronic cholecystitis with severe dysplasia	2 (2.6)
Neoplastic	
Adenocarcinoma	9 (11.8)
Total	19

Table 3: Depicts association of HER2/neu expression with different gallbladder lesions

Lesion category	HER2 neu index		Total (%)	Statistical test χ^2	*P*
	Positive (%)	Negative (%)			
Nonneoplastic	0	57 (100)	57 (100)	39.844	0.001
Preneoplastic	0	10 (100)	10 (100)		
Neoplastic	5 (55.55)	4 (44.45)	9 (100)		
Total	5 (6.5)	71 (93.5)	76 (100)		

χ^2 : chi-sqaure test

Table 4: Depicts association of Ki-67 expression with different gallbladder lesions

Lesion category	Ki-67 (labeling index)		Total (%)	Statistical test χ^2	*P*
	Positive (%)	Negative (%)			
Nonneoplastic	0	57 (100)	57 (100)	44.046	0.001
Preneoplastic	4 (40)	6 (60)	10 (100)		
Neoplastic	7 (77.8)	2 (22.2)	9 (100)		
Total	11 (14.4)	65 (85.6)	76 (100)		

χ^2 : chi-sqaure test

neu positive expression in 23% of GBC cases with entirely absent HER2/neu expression in nonneoplastic gallbladder lesions (control group).[15] Contrasting findings were observed in research conducted by Zhou *et al*. as they observed HER2/neu positive expression in 70.7% of GBC conditions.[16] With respect to clincopathological parameters, HER2/neu positive expression was observed prominently in females, older age, and subjects clinically presenting with weight loss with respect to age and gender. The findings were similar to the findings observed in the study done by Pujani *et al*. Regarding Ki-67, 57.8% (11/19) of the neoplastic group demonstrated positive Ki-67 expression, with completely absent expression in the nonneoplastic group.[14] The current research demonstrated a substantial association between Ki-67 expression and age of subjects with ($P = 0.001$) and findings were comparable to the research done by Doval *et al*.[17] The study done by Doval *et al*. demonstrated positive Ki-67 expression in 55% of GBC cases with completely absent expression in the nonneoplastic group, which is similar to findings observed in current research.[17] Pujani *et al*. also observed Ki-67 expression in 60% of neoplastic cases, which is nearly similar to the findings in the current study.[14] Ki-67 expression was prominently noted in females, older age and subjects clinically presenting with jaundice. With progressive advancement in targeted treatment modalities which acts against numerous cell adhesion molecules with coexistent HER2/neu overexpression in GBC validates these two HER2/neu and KI-67 as potential candidate for this targeted treatment. As these markers demonstrated substantial difference between neoplastic and neoplastic groups, it signifies that they play crucial role in tumorigenesis in GBC cases.

Limitations of study

It was a unicentric study with a limited sample size.

Conclusions

HER2/neu, Ki-67 overexpression was predominantly observed in neoplastic gallbladder lesions as compared with nonneoplastic (control group) with no substantial association among clinicopathological parameters with the exception of age and gender. Based on the overexpression noted in neoplastic cases, HER2/neu can act as a potential site for targeted treatment, especially in GBC cases.

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Conflicts of interest

There are no conflicts of interest.

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